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Tolerance of *Serpula lacrymans* to copper-based wood preservatives ☆

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Abstract

Serpula lacrymans, the dry rot fungus, is considered the most economically important wood decay fungus in certain temperate regions of the world, namely northern Europe, Japan, and Australia. Previously, copper-based wood preservatives were commonly used for pressure treatment of wood for building construction, but some decay fungi are known to be copper tolerant. In this study, soil-block tests were undertaken to clarify the effect of copper, copper citrate, and alkaline copper quaternary-type D (ACQ-D) on the decay capabilities of *S. lacrymans* compared with an alternative wood preservative not containing copper. Twelve isolates of the dry rot fungus *S. lacrymans* and four other brown-rot species were evaluated for weight loss on wood treated with 1.2% copper citrate, 0.5% ACQ-D, and 0.5% naphthaloylhydroxylamine (NHA). Eleven out of 12 isolates of *S. lacrymans* were shown to be tolerant towards copper citrate. The ACQ-D and NHA preservatives, on the other hand, were both effective against the dry rot isolates.

Keywords: *Serpula lacrymans*; Dry rot; Copper tolerance; Copper citrate; ACQ-D; NHA

1. Introduction

Copper tolerance can be defined as the ability of an organism to grow and thrive in the presence of copper ions. In recent years, the focus on copper-based wood preservatives has increased following concerns about the environmental effect of chromium and arsenic and the resulting restrictions of chromated copper arsenate (CCA) use. Copper-based preservatives have been widely and successfully used for more than a century (Humar et al., 2001) because copper exhibits good biocidal activity (Nicholas and Schultz, 1997). It is still the most frequently used biocide for wood preservation, but a major requirement of any formulation of copper-based wood preservative is efficacy against copper-tolerant fungi (Peek et al., 1993; Nicholas and Schultz, 1997). Copper-based preservatives have been used to protect wood against wood decaying fungi, especially the dry rot fungus, *Serpula lacrymans*. This fungus has become an increasing hazard in houses in many temperate regions of the world, and it is of great economic importance to be able to prevent damage by this fungus.

S. lacrymans has previously been tested for copper tolerance with contradicting results. Schmidt and Moreth (1996) tested the influence of increasing copper concentrations on mycelial growth when the fungus was grown on malt agar. *S. lacrymans* was only able to grow at the lowest concentration of 1 mM Cu, and no growth

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was reported at a concentration of 5 mM Cu. Others found low levels of copper in the wood were able to stimulate decay in of some strains of *S. lacrymans* and that the relative copper tolerance of strains varied with the formulation of the preservative (Woodward and De Groot, 1999). This variation in formulation was tested using both copper citrate (CC) and alkaline copper quaternary-type D (ACQ-D). Others (Tsunoda et al., 1997) found a single isolate of *S. lacrymans* not to be copper tolerant. It was possible for them to inhibit the growth of *S. lacrymans* in agar with a copper concentration of 1250–2500 ppm. The decay ability of *S. lacrymans* in wood was completely suppressed at a retention of 2.0 kg m^{-3} copper (II) sulphate.

A relationship between copper tolerance and oxalic acid production has been implicated, because of copper oxalate crystal formation in decayed wood (Murphy and Levy, 1983). Rabanus (1933) was the first to link the production of oxalic acid with copper tolerance, and he demonstrated precipitation of copper oxalate when fungi were grown on an agar plate containing copper sulphate. Rabanus (1933) and Shimazono and Takubo (1952) suggested that tolerance of brown-rot fungi is linked to oxalic acid production, which presumably precipitates copper into the insoluble form of the oxalate, rendering the copper metabolite inert. Both studies concluded that lowering of the pH by oxalic acid had more to do with copper tolerance than low solubility of copper oxalate. Since then, copper tolerance has been linked to oxalic acid production in a number of fungal species such as *Wolfiporia cocos* and *Poria placenta* (Sutter et al., 1983; Clausen et al., 2000; Green and Clausen, 2005). To the contrary, Schmidt and Moreth (1996) found that despite considerable oxalic acid production by the brown-rot fungus *Antrodia sinusa,* it did not show copper tolerance. Furthermore, Collet (1992) reported that isolates of the related species, *Antrodia vaillantii* differed significantly in their tolerance to copper. Variation in preservative tolerance among isolates of individual fungal species has not been adequately investigated (Collet, 1992). The same may be true for *S. lacrymans*. Green and Clausen (2003) found rapid induction of oxalic acid (exceeding 600 μmol g^{-1}) by 11 copper-tolerant brown-rot fungi that effectively decayed copper citrate-treated southern pine blocks. This mechanism contributes to the detoxification of copper in copper-treated wood, which enables fungi to tolerate environments containing high concentrations of toxic metals. Since copper oxalate is insoluble, copper in this form has a greatly reduced inhibitory effect on fungal growth (Woodward and De Groot, 1999; Humar et al., 2001).

The objective of this study was to evaluate a population of *S. lacrymans* (Wulf.: Fr.) Schroeter for copper tolerance to ammoniacal CC and ACQ-D in comparison to an alternative wood preservative, *N′N*-naphthaloylhydroxylamine (NHA). This is the first report to examine the effect of NHA on *S. lacrymans*.

Copper citrate has been used as a preservative model to study copper-tolerant fungi in wood because it lacks co-biocides that are found in most copper-based preservatives (Clausen et al., 2000). For the purpose of studying copper tolerance, the presence of co-biocides, such as chromium and arsenic, could confound the results (De Groot and Woodward, 1998). ACQ-D, which contains a quaternary co-biocide, is included in this study because it is one of several copper-containing wood preservatives that have been developed in recent years as a replacement for CCA (Green and Clausen, 2005). NHA is a water-soluble calcium capture agent (Green et al., 1997, 2002) and is a relatively benign compound with lower toxicity in aquatic environments to *Daphnia* sp. than copper (around 130 times less) (Crawford and Green, 2004). Therefore, it could become a very important alternative to copper-based preservatives if it proves efficacious.

2. Experimental methods

2.1. Fungal cultures

Twelve isolates of *S. lacrymans*, two isolates of *S. himantioides*, and one isolate each of *Tyromyces palustris*, *Gloeophyllum trabeum*, and *Postia placenta*, provided by Technological Institute, Denmark; Håvard Kauserud, Oslo University, Norway; and Forest Products Laboratory, Madison, WI, USA, were maintained on 2% malt extract agar (Difco Laboratories, Detroit, Michigan, USA) (Table 1).

2.2. Decay test

Southern yellow pine (SYP) sapwood blocks (10 × 10 × 10 mm) and SYP sapwood feeder blocks (3 × 28 × 35 mm) were conditioned to 6% equilibrium moisture content and weighed. The blocks were then vacuum-treated with 0.5% ACQ-D, 1.2% ammoniacal CC, or 0.5% NHA (Table 2). Preweighed blocks were submerged in respective treating solutions and subjected to a vacuum of 165.5 kPa gage pressure twice for 20 min each. Blocks were weighed, dried in a fume cupboard overnight, returned to the conditioning room for 1 week, and reweighed. The blocks were subjected to *T. palustris, G. trabeum, P. placenta,* 2 isolates of *S. himantioides,* and 12 isolates of *S. lacrymans* (Table 1) in a soil-block test following the guidelines of American Wood Preservers' Association Standard E10-01 (AWPA, 2003a).

All blocks were steam-sterilized for 30 min without pressure prior to setting them onto the wood block feeders covered with mycelium of the test fungus. Soil

Table 1
Strains used in soil-block tests

Fungal isolate	Fungal species	Origin	Growth temperature (°C)
Bb 29	*S. lacrymans*	Belgium: Baisy-Thy	20
Sl199	*S. lacrymans*	Japan: Asahikawa	20
Sl200	*S. lacrymans*	Poland: Warsaw	20
Sl202	*S. lacrymans*	France: Xylochimie	20
Sl207	*S. lacrymans*	England: Rothesay	20
Sl209	*S. lacrymans*	Germany: Velbert	20
Sl210	*S. lacrymans*	Germany: Krefeldt	20
Sl216	*S. lacrymans*	Norway: Fannrem	20
Sl217	*S. lacrymans*	Norway: Oslo	20
Sl219	*S. lacrymans*	Finland: Helsinki	20
Sl221	*S. lacrymans*	Finland: Helsinki	20
BamEbers 315	*S. lacrymans*	Germany: Eberswalde	20
ATCC 11485	*S. lacrymans*	USA: North Carolina, Asheville	20
ATCC 36335	*S. himantioides*	USA: Mississippi, Gulfport	20
Sh100	*S. himantioides*	Germany: Wilsede	27
Mad 617	*G. trabeum*	USA: Wisconsin, Madison	27
Mad 698	*P. placenta*	USA: Maryland	27
TYP 6137	*T. palustris*	Japan: Uji	27

Table 2
Wood preservatives and retentions

Preservative treatment	Copper composition	Active ingredient (w/w%)	ICP[a] calculated retention ($mg\,g^{-1}$)	Calculated retention ($kg\,m^{-3}$)
Alkaline copper quat-type D (ACQ-D)	66.7% CuO	0.5 Cu; 33% DDAC[b]	1.4 Cu	1.3 ACQ-D
Copper citrate (CC)	62.3% CuO	1.2 Cu	4.6 Cu	4.7 CC
CC + $CaCl_2$	62.3% CuO	1.2 Cu, 1.0 Ca	5.8 Cu, 3.2 Ca	6.0 CC
N′N-naphthaloylhydroxylamine (NHA)	0% CuO	0.5% NHA	—	—

[a]ICP, inductively coupled plasma spectrometry (AWPA, 2003b).
[b]DDAC, didecyldimethylammonium chloride.

bottles were incubated at 20 or 27 °C (Table 1) at 70% relative humidity (RH) for 10 weeks to optimize conditions for *Serpula* sp. and the other wood decay fungi, respectively. Five replicates of treated and untreated blocks for each fungal isolate were harvested after incubation for 1, 2, 4, 6, 8 or 10 weeks. Blocks were removed from bottles, brushed free of mycelium, weighed, dried at 60 °C for 24 h, weighed, conditioned to 70% RH, and reweighed. Percentage of weight loss was calculated from the conditioned weights before as well as after decay testing.

3. Results and discussion

The decay capacity of *S. lacrymans* in untreated wood was compared with that in copper citrate-treated wood (Table 3). Only the 10-week incubation weight losses are discussed in this paper. Mean percentage weight loss ($n = 5$) in treated wood is ranked from highest to lowest and ranges from 46.68 to 9.81%.

Three isolates decayed copper citrate-treated and untreated wood to the same degree (Sl 221, Sl 200, and Sl 199). One isolate (Bam Ebers 315) was significantly inhibited by CC (78.2% less weight loss compared with untreated controls), and seven isolates were moderately inhibited by CC (12.5–38% less weight loss in treated wood than in untreated controls). In Bb 29, CC treatment of the wood seemed to increase the decay capacity. A 21.50% average increase in weight loss for copper citrate-treated wood was seen compared with untreated wood (Table 3). A 95% confidence interval analysis found this increase in weight loss not to be statistically significant.

For comparison, decay capacity is shown for *S. himantioides*, also known as the wild dry rot fungus, *T. palustris* and *P. placenta*, which are known copper-tolerant decay fungi, and *G. trabeum,* a copper-sensitive decay fungus that is a nonaccumulator of oxalic acid (Table 1). The *S. himantioides* isolate Sh100 showed no difference between CC-treated and untreated wood for decay capacity, whereas ATCC 36335 was moderately

Table 3
Decay capacity of 17 brown-rot fungal isolates after 10 weeks growth on untreated and copper citrate, alkaline copper quat-type D (ACQ-D), and *N′N*-naphthaloylhydroxylamine (NHA) treated southern pine

Fungal isolate	Mean weight loss (%)			
	Treated			Untreated
	Copper citrate	ACQ-D	NHA	Control
S. lacrymans				
Sl 221	46.7±5.3	1.6±1.9	7.8±6.5	46.2±5.2
Bb 29	38.3±5.8	2.5±1.9	6.1±3.0	31.5±13.7
ATCC 11485	33.1±10.8	2.5±1.4	8.1±1.4	38.1±7.1
Sl 219	32.9±18.7	0.4±2.8	5.2±0.6	53.1±6.9
Sl 216	32.3±14.0	0.4±0.4	24.7±12.4	44.6±12.5
Sl 202	31.6±3.2	4.8±6.7	12.3±11.2	44.0±10.6
Sl 200	29.0±11.5	1.2±0.4	3.2±0.7	31.4±9.2
Sl 217	29.0±9.9	0.8±0.6	20.0±6.2	42.2±5.5
Sl 207	29.0±7.9	5.3±9.6	5.8±4.5	33.1±8.6
Sl 199	27.7±6.5	1.2±0.4	4.3±4.8	27.5±4.2
Sl 209	27.4±11.6	0.5±0.9	8.0±6.2	38.7±5.8
Bam Ebers 315	9.8±6.5	2.9±1.0	5.2±1.7	45.0±9.0
S. himantioides				
Sh100	45.9±10.5	18.7±4.7	18.8±14.0	47.7±3.8
ATCC 36335	28.4±15.3	0.2±0.4	21.3±12.6	38.7±4.5
P. placenta				
Mad 698	57.0±11.5	1.8±0.4	3.7±2.5	68.5±1.9
T. palustris (Fomitopsis palustris)				
TYP 6137	39.6±9.4	19.7±12.3	44.3±4.8	48.2±6.4
G. trabeum				
Mad 617	4.8±7.2	0.1±0.1	8.8±3.7	59.4±8.0

inhibited by CC. *T. palustris* and *P. placenta* were also moderately inhibited by CC in this test. *G. trabeum* was significantly inhibited.

In general, the ACQ-D- and NHA-treated wood showed markedly better inhibition of the *S. lacrymans* isolates, which demonstrates the role of the alkaline quats in inhibiting wood decay. For the isolates grown on ACQ-D-treated wood, the decrease in decay ranged from 84% to 99%. For the other isolates, there was the same tendency. All weight losses in the treated wood ranged from 59% to ~100% less than in the untreated wood.

The NHA-treated wood showed a 45–90% decrease in decay by Sl 216 and Sl 219, respectively. The other isolates showed the same pattern towards NHA-treated wood, except the *T. palustris* isolate. The *T. palustris* isolate TYP-6137 showed almost no inhibition in decay (8.1% less decay in blocks treated with NHA).

4. Conclusion

S. lacrymans and *S. himantioides* display a considerable copper tolerance towards the wood preservative, CC. Of the 12 *S. lacrymans* isolates tested, only one (BAM-Ebers 315) was significantly inhibited by copper, whereas 10 isolates showed moderate to no inhibition by copper. Wood blocks treated with CC showed an average of 31% weight loss when challenged with isolates of *S. lacrymans*. In comparison, the untreated blocks had an average decay of 40% after 10 weeks of incubation. Thus, the treatments with CC caused an average decrease in decay by 21%. This cannot be considered an efficient wood protection agent against dry rot decay.

The two alternative wood protection solutions tested in this study (ACQ-D and NHA) were much more effective in preventing wood decay by the test fungi than CC as previously described (Green and Clausen, 2005). The ACQ-D-treated wood blocks showed an average of only 2%±2% weight loss after 10 weeks of incubation. The wood blocks treated with NHA solution showed an average of 9% weight loss after 10 weeks of incubation. In these blocks, the variance was higher between the isolates, ranging from 3% to 25% weight loss in the wood blocks. The *S. himantioides* isolate (Sh100) and *T. palustris* seemed to be fairly aggressive no matter what treatment the wood blocks were exposed to.

The results from this study show that caution should be taken when choosing wood protection agents now that traditional biocides are no longer acceptable. The soil-block tests showed that most isolates of *S. lacrymans* are not inhibited by copper alone. The alternative solutions, which do not rely solely on copper, give much better resistance to dry rot decay in wood.

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